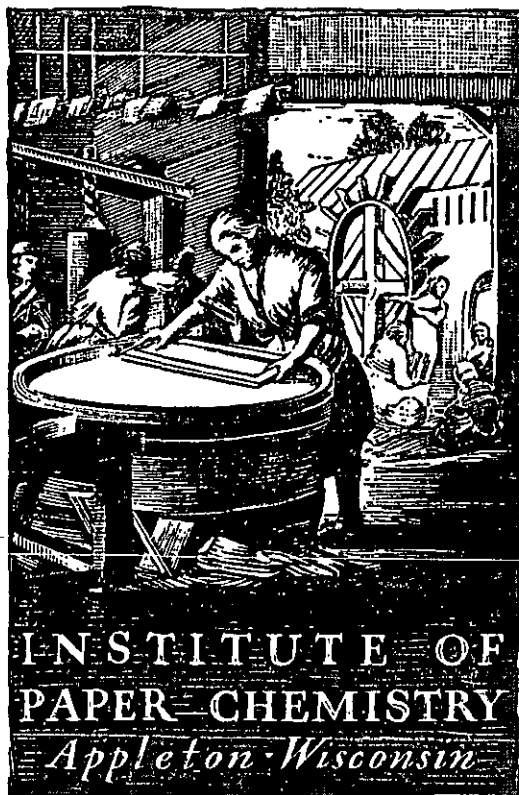


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INHIBITION OF BANANA ROT ORGANISMS

Project 1108-19

Progress Report One
to
FOURDRINIER KRAFT BOARD INSTITUTE, INC.

July 17, 1957

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

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INTRODUCTION

The physical structure and chemical nature of the banana fruit makes it very susceptible in shipment to spoilage resulting from mechanical abrasion and storage rot. The latter results from the action of the fungi and manifests itself as stem-end rot or crown rot. The banana fruit is very susceptible to fungus attack and is naturally contaminated both in the field and during subsequent handling procedures by a variety of organisms capable of causing storage rot. It is anticipated that the packaging of bananas in corrugated fiberboard boxes will materially reduce mechanical abrasion and damage due to "rough handling". Present methods of controlling the deterioration or spoilage by fungi consist of rapid and efficient handling plus refrigeration. Previous experience in packaging of fruit in fiberboard boxes has been that the increased humidity within the box enhances mold sporulation and growth. There is little to suggest that this will not also be the case with bananas.

To prevent severe decay losses of bananas during shipment in fiber boxes, the use of a fungicidal or fungistatic agent may be required. It is the purpose of this study to examine the suitability of various chemical agents in this respect and to determine how they can best be applied to prevent fungus attack on bananas.

EXPERIMENTAL PROCEDURE AND RESULTS

A long list of fungi has been noted in the literature as being, to varying degrees, responsible for decay losses of bananas during storage. The more important of these fungi includes the species Gleoesporium musarum, Botryodiplodia theobromae, and Thielaviopsis paradoxa. The American Type Culture Collection, Washington, D. C. was contacted for samples of these cultures. It was desired to obtain cultures of these fungi in order to be able to grow a sufficient quantity of each for subsequent inoculation of bananas for the purpose of testing the degree of control obtained with various fungicidal or fungistatic agents. We were unable to obtain the conidial stage fungi G. musarum and T. paradoxa; however, cultures of their perfect stages Glomerella cingulata and Ceratocystis (Ceratostomella) paradoxa, respectively were received.

In addition to sending for cultures of known banana rot organisms, isolations have been made from several specimens of banana forwarded by Mr. Loeb from Detroit showing crown rot (alias finger stem rot). Isolation was made on four types of Difco prepared media (corn meal, malt, potato dextrose, and Sabourauds agar) and at pH levels of 4.5 and 5.5. From these isolations seven and possibly nine genera of fungi have developed on the various agar media. Microscopic examination indicates the following genera are represented: Cephalosporium, Cladosporium, Helminthosporium, Penicillium, Rhizopus and Trichoderma. Work is now in progress to obtain pure cultures of the organisms which have been isolated and further study will be required for positive identification if such is necessary.

PROGRAM IN PROGRESS

The basic objective of the current activity is namely to select a biostatic agent which will prevent decay losses of bananas in storage or at least maintain such losses at an economic level. The problem at hand is to develop techniques which will give the desired information and in a reasonable length of time.

First, there is the selection of candidate fungistats to be included in the test. An ideal material will have to satisfy very strict requirements. ~~It must effectively inhibit a broad spectrum of organisms,~~ it must not alter the appearance or taste of the fruit, it must not be a hazard to those subsequently handling or eating the fruit, and it must be economically feasible. The prospects of finding such an ideal material in a short period of time are not too bright. Consequently it may be necessary to use two or more agents to accomplish the above objective.

There are two methods of application under consideration--dip treatment and vapor treatment. The vapor treatment has certain advantages in the areas of application and toxicology; therefore, emphasis will be placed on this method of treatment. Suggested compounds are biphenyl, methyl dichlorosuccinate, n-butyl dichlorosuccinate, and ammonia. Salts which decompose and release ammonia slowly will be tested for this particular treatment. Compounds which may prove effective as dip treatments include orthophenyl phenol, sorbic acid, and sodium and calcium propionates. Obviously, there are many other compounds which can be tested; however, those mentioned have all shown a certain degree of promise in other commercial applications.

Also to be selected are the fungi species to be used as test organisms. As previously noted, a large number of different types of fungi are capable of damaging bananas in storage. They can be divided into three groups--(1) primary parasites, (2) wound parasites, and (3) secondary invaders. The first group are capable of attacking the fruit when conditions for growth are favorable. The second group will initially attack the fruit at some damaged site and proceed beyond this point into undamaged tissue. The third group includes a very wide range of saprophytic fungi which follow the growth of the parasitic fungi and increase the over-all damage. Thus, the secondary invaders do not manifest themselves until after primary parasites or wound parasites have been active.

It is, therefore, only necessary to prevent the initial attack by the first two groups named to reduce storage losses. The secondary invaders, however, cloud the picture when making isolations from moldy banana specimens such as has been done in our laboratory. In order to select the pertinent species from these isolations, tests will be required to determine which are parasitic and which saprophytic. These tests are complicated by the fact that conditions for parasitism are commonly very exacting for each type of fungus. The moisture content of the atmosphere, the site and extent of an injury, the natural resistance of a particular inoculated banana, and degree of ripeness are examples of factors determining whether or not a successful infection can be obtained. Another important microbiological aspect of parasitism is that frequently when a test organism is grown on artificial agar media, it loses the ability to infect its natural host--i.e., in this case the banana. This may be true of known parasitic organisms obtained from culture collections.

It would be possible to run inhibition tests on selected fungi in agar media and side-step the problem of parasitism. However, this makes the validity of the results in terms of commercial usage subject to question and it is preferable to run the tests using bananas as the test substrate provided a high percentage of infection of untreated controls can be achieved. Our approach, therefore, has been to make initial tests on the organism B. theobromae and on the perfect stage cultures of G. musarum and T. paradoxa to determine their parasitic activity. Also, the organisms we have isolated will be tested in the same manner but rather than spend the time it would require to prove with certainty their individual parasitic nature, it was decided to use these organisms as a combined inoculum. These tests will be conducted by inoculating green bananas and allowing them to ripen at 68°F. in desiccator jars. Potassium hydroxide will be used to remove carbon dioxide evolved by the fruit.

Assuming the above inoculations are successful, the next step will be to screen the test materials using the above procedures of inoculation and incubation. Once the relative merits of the various chemical agents and methods of application are measured, storage trials will be in order.

The time required for the above study will be relatively long due to the fact it will require several weeks for each transfer of fungus to show growth. The optimum conditions provided by the desiccator jars, temperature, etc. should speed the test considerably over normal commercial storage conditions but immediate results are not possible. The reasons for not launching storage trials initially, however, involves not only time but the possibility that infection may be difficult and if low

would mean a negative result for the entire experiment. In previous work of this nature, involving storage of fruit, it has proven wisest to move slowly into storage trials.

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